Investigation of physiological activity and mixture effects of G protein-coupled receptor-acting pharmaceuticals in wastewater(Abstract_要旨)

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（ 続紙 1 ）

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論文題目	Investigation of physiological activity and mixture effects of G protein-coupled receptor-acting pharmaceuticals in wastewater (下水中に存在する G タンパク質共役型受容体に作用する医薬品の生理活性と複合作用に関する研究)		

Chapter 1 describes the background, objectives and structure of this thesis.

Chapter 2 shows the detailed background of this thesis. Firstly, the adverse effects of pharmaceuticals in public waters on aquatic organisms are introduced. Then, limitations of current methods for environmental monitoring and toxicity testing of pharmaceuticals are discussed. Because most pharmaceuticals act on receptors to bring about the effects, current methods and endpoints are inappropriate to measure their physiological activities. Then, the necessity of the method to detect the physiological activity of G Protein-Coupled Receptor (GPCR) acting pharmaceuticals is discussed. Finally, advantages of *in vitro* Transforming Growth Factor-α (TGFα) shedding assay on measurement of physiological activity of GPCR-acting pharmaceuticals is explained.

In **Chapter 3**, recovery of antagonistic activity with solid phase extraction(SPE) against 5 GPCRs consisting of Angiotensin(AT1), Dopamine(D2), Acetylcholine(M1), Adrenergic(β1) and Histamine(H1) in pure water and secondary effluent was tested by the *in vitro* TGFα shedding assay. Antagonistic pharmaceuticals for AT1, D2, M1, β1 and H1 receptors such as Valsartan, Sulpiride, Pirenzepine, Metoprolol and Diphenhydramine, respectively, were selected. The results indicated that, for AT1, β1 and H1 receptors, activity measured by the TGFα shedding assay was directly comparable with antagonist concentration of chemical analysis. For D2 and M1 receptors, when the activity was at several hundred ng/L level, the activity was directly comparable to antagonist concentration of chemical analysis. However, when the activity was at several thousand ng/L level, the loss of activity could be up to 40%. Therefore, the TGFα shedding assay could quantitatively measure the antagonistic activity of GPCR-acting pharmaceuticals under the above conditions.

In **Chapter 4**, secondary effluent and/or final effluent of three wastewater treatment plants (WWTPs) were collected in the UK and Japan, which were in different areas. Samples were concentrated by SPE and analyzed by the TGFα shedding assay. Strong antagonistic activity against AT1, D2, M1, β1 and H1 receptors was detected in the UK for the first time. Agonistic activity was also detected in the UK for the first time. Detected activity was compared with the concentrations of current targeted pharmaceuticals, the results indicated that other antagonists on the same GPCR receptors exist in secondary or final effluent.

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In **Chapter 5**, for AT1, D2, M1, ß1 and H1 receptors, some pharmaceuticals were selected on the basis of the consumption ranking of GPCR-acting pharmaceuticals in the UK in 2014. For each GPCR, binary mixtures of antagonists were prepared and analyzed by the TGFα shedding assay. The activity of binary mixtures was as potent as that of single pharmaceutical, which indicated that pharmaceuticals with antagonistic effects on the same GPCR receptor show an additive effect. Concentration addition model was applied to predict the activity of current antagonistic pharmaceuticals against ß1 receptor, then the predicted activity was compared with measured activity. The results indicated that propranolol and atenolol are prioritized among ß1 antagonists in the UK.

In **Chapter 6**, binary mixtures of antagonist and agonist were prepared at fixed molar ratio, then the mixtures were analyzed by the TGFα shedding assay. Increasing the proportion of agonist to antagonist in the mixture, maximum inhibition was suppressed, and the IC_{25} became larger. These indicated that agonist and antagonist would compete. Secondary effluent samples in the UK and Japan were fractionated by increasing concentration of methanol in MilliQ water solution (20%, 40%, 60%, 80% and 100%). Unfractionated samples were prepared in parallel. All the fractionated and unfractionated samples were analyzed by the TGFα shedding assay. Antagonistic activity and agonistic activity were detected in different fractions. Finally, activity of each fraction was compared with activity of unfractionated sample. In UK samples, for M1 and ß1 receptors, agonistic activity of 60% fraction was below limit of detection, however, antagonistic activity of 60% fraction was higher than activity of unfractionated sample. In Japanese samples, agonistic activity of 60% fraction with D2 receptor was below limit of detection, however, antagonistic activity of 60% fraction was higher than activity of unfractionated sample. These results indicated that agonists and antagonists co-exist and compete each other in secondary effluent of the UK and Japan.

In **Chapter 7**, future recommendations are proposed. Physiological activity of GPCR-acting pharmaceuticals should be studied in raw wastewater. Removal efficiency of activity between different treatment processes should be studied. The main causative compounds for each GPCR should be identified in the future. Priority of pharmaceuticals for *in vivo* testing should be proposed.

Chapter 8 describes summary of results and conclusions for each chapter.

（続紙 2 ）

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（論文審査の結果の要旨）

低濃度で存在する水中の残留医薬に生物が曝露される場合に、その生理活性の影響が懸念されている。近年、G タンパク質共役型受容体（GPCR）に作用する医薬品に対して、トランスフォーミング増殖因子アルファ(TGFα)切断アッセイが開発され、環境水への適用に関心がもたれている。本研究は、TGFα切断アッセイの下水処理水の生理活性への適応性、GPCR への作用を持つ医薬品の複合作用を検討したものである。主要な成果は下記の通りである。

(1) 固相抽出法を用いて、下水処理水からのアンタゴニスト型医薬品を濃縮する妥当性を検討した。高血圧治療薬の標的である Angiotensin 受容体(AT1)， 自律神経系薬、高血圧治療薬、気管支拡張薬の標的である Adrenergic 受容体(β1)、アレルギー治療薬の標的である Histamine 受容体(H1)、統合失調症治療薬の標的である Dopamine 受容体(D2)、自律神経系薬、気管支拡張薬の標的である Acetylcholine 受容体(M1)のアンタゴニストである 5 種類の医薬品を下水処理水に標準添加し、生理活性を測定した。その結果、添加したアンタゴニストは数百 ng/L レベルでは 80%以上の生理活性が得られ、この方法が下水処理水の生理活性の測定に適用可能であることが示された。

(2) 英国の下水処理水を対象に AT1、 D2、β1、M1、H1 受容体アンタゴニスト活性を測定した。日本と同様に、英国でもμg/L レベルで生理活性が検出された。D2、M1、β1 受容体のアンタゴニスト活性は、それぞれのアンタゴニストである sulpiride、pirenzepine、metoprolol の濃度に相当する生理活性よりも大きかった。下水処理水にはこれら以外にも同じアンタゴニスト作用を持つ物質が存在し、相加的に作用していることが示唆された。

(3) 英国で多用されている AT1、D2、M1、β1、H1 受容体のアンタゴニスト医薬品から 2 成分混合物を作成し、受容体ごとの生理活性作用の複合作用を調べた。その結果、同じ受容体への作用機序をもつ医薬品は、相加性を持つことが示唆された。

(4)アゴニストとアンタゴニストの 2 成分混合物を作成し、TGFα切断アッセイで生理活性を評価した。その結果、アゴニストとアンタゴニストは競合することが示唆された。また下水処理水を異なるメタノール濃度の水溶液で分画すると、アゴニスト作用のない分画成分のアンタゴニスト作用は、未分画試料よりも大きくなり、下水処理水でも、実際にアゴニストとアンタゴニストが競合していることが示唆された。したがって、TGFα切断アッセイは両者の総合的な評価となっている。

以上のように本論文は、低濃度で存在する水中の残留医薬品のうち、GPCR への作用を持つ医薬品の生理活性を TGFα切断アッセイによって評価した研究であり、下水処理水の生理活性への適応性、GPCR への作用を持つ医薬品の複合作用を検討したものであり、その可能性・有効性についての研究成果をまとめたものである。この成果は残留医薬品類の生態系影響を評価し、生理活性作用の削減技術を評価する上で重要な成果となると考えられ、学術上、実際上寄与するところが少なくない。よって、本論文は博士（工学）の学位論文として価値あるものと認める。また、平成 29 年 8 月 22 日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。